Vol. 120, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 16, 1984 Pages 232-236

AMINO-TERMINAL AMINO ACID SEQUENCE OF THE NONSPECIFIC PHOSPHOLIPID EXCHANGE PROTEIN FROM BOVINE LIVER

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Received March 5, 1984

The amino-terminal amino acid sequence of the nonspecific phospholipid exchange protein from bovine liver has been determined. The first 52 amino-terminal residues in the sequence were identified. The sequence determined failed to show statistically significant homology to any previously published protein sequence. However, a stretch of 12 amino acids at the end of the sequence displays homology to the phosphatidylcholine-specific phospholipid exchange protein.

Phospholipid exchange proteins (PLEPs)[1] are cytosol proteins which extract a phospholipid molecule from one membrane and insert it into another membrane (1-3). Phospholipid exchange has been demonstrated in a wide variety of eukaryotic cells, and a number of PLEPs have been purified to homogeneity. Different PLEPs vary in their phospholipid specificity, and several specific PLEPs are often found in one and the same tissue (4-12).

The most thoroughly investigated PLEP is the phosphatidylcholine-specific one in bovine liver, whose amino acid sequence has been elucidated (13). Another exchange protein found in liver is the so-called nonspecific PLEP. This is a small basic protein with a particularly broad phospholipid specificity. It has been purified from rat liver (9, 14) and from bovine liver (12, 15). Here we report the amino-terminal amino acid sequence of the nonspecific PLEP from bovine liver.

MATERIALS AND METHODS

Purification of the nonspecific PLEP from bovine liver. The nonspecific PLEP of bovine liver was purified essentially as described (12). However, the final step in the published protocol, i.e. an octylagarose chromatography, was omitted since we encountered significant losses of material due to aggregation of the protein. Instead, the concentrated heat supernatant was dialyzed against the low ionic strength buffer and then applied

[1] Abbreviations used: PLEP - phospholipid exchange protein

0006-291X/84 $1.50

to the CM-cellulose column previously used to subfractionate the highly purified nonspecific PLEP into the two forms CMI and CMII (12). All contaminating proteins eluted at an early position in the gradient. Therefore, the most basic form of the nonspecific PLEP was obtained in a highly purified form. The high degree of purity of the isolated PLEP was ascertained by high resolution SDS-polyacrylamide gel electrophoresis (16) and by amino acid analysis.

Amino acid analysis. The nonspecific PLEP was reduced and alkylated as described (17). Samples were hydrolysed for 24 hours in 6 M HCl containing 0.1% phenol, and analysed on a Beckman 121 M amino acid analyzer. Tryptophans and CM-cysteines were not determined. To facilitate comparisons with the data in ref. 12, nearest integer values were calculated for a total of 122 non-cysteine, non-tryptophan residues.

Amino acid sequence determination. The reduced and alkylated protein (100 nmol) was sequenced in a Beckman 890 C liquid phase sequencer (18). Phenylthio-hydantoin-amino acids were analysed by reversed phase high pressure liquid chromatography (19). Calculations of yields were corrected for background and carry-over. When the same residue occurred in adjacent positions, the combined yield was divided equally on these positions.

RESULTS AND DISCUSSION

Amino acid composition of the nonspecific PLEP. The amino acid composition of the bovine liver nonspecific PLEP, isolated as detailed above, is given in Table I. The data are very similar to those in ref. 12. In the present study we did not attempt to

TABLE I

Amino acid composition of the bovine liver nonspecific PLEP

Amino acid	mol %	Number of residues[a]	Number of residues[b]	
			CMI	CMII
Lysine	13.6	17	17	17
Histidine	0.4	0	0	1
Arginine	0.7	1	0	1
Aspartic acid	12.4	15	16	16
Threonine	4.2	5	5	5
Serine	4.9	6	5	5
Glutamin acid	10.6	13	13	13
Proline	3.9	5	4	4
Glycine	11.1	14	15	15
Alanine	7.6	9	9	9
Valine	7.0	9	8	8
Methionine	4.2	5	5	5
Isoleucine	4.2	5	5	5
Leucine	9.2	11	11	11
Tyrosine	0.3	0	0	0
Phenylalanine	5.4	7	7	7
Cysteine	N.D.		4	4
Tryptophan	N.D.		1	1

[a] Nearest integers calculated on the basis that the amino acids determined comprise a total of 122 residues.

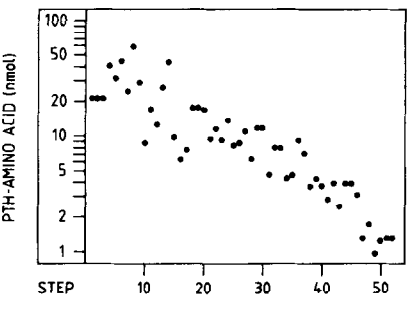
[b] Data from ref. 12.

isolate the two distinct forms of the protein, CMI and CMII, that differ inasmuch as CMII contains one mole of histidine and one mole of arginine per mole of protein (12). Our preparation contained approximately one mole of arginine but less than half a mole of histidine per mole of protein (Table I). Thus, it occupies an intermediate position between the CMI and CMII forms.

A possible explanation for the differences between CMI, CMII and our preparation is that the protein may undergo limited exoproteolysis, which is a common cause of microheterogeneity in protein preparations. Since neither histidine nor arginine is present at the amino-terminus (see below), the exoproteolysis would have to occur at the carboxy-terminal end of the molecule.

Amino-terminal amino acid sequence of the nonspecific PLEP. The purified nonspecific PLEP was subjected to amino-terminal amino acid sequence analysis. The first 52 residues in the amino-terminus of the protein were unambiguously identified (Fig. 1). A search for homology with published protein sequences was carried out using the SEARCH program (20) and the National Biomedical Research Foundation database (updated June 1, 1983). No significant homology to any previously sequenced protein was detected, suggesting that the nonspecific PLEP does not belong to any of the hitherto described protein families.

In a further search for homology the RELATE program (20) was used to compare the sequence of the nonspecific PLEP to the sequences of various other small intracellular lipid-binding proteins. These comparisons failed to reveal any homology to the Z-protein (21) or to the cellular retinol and retinoic acid binding proteins (22, 23). However, a comparison to the phosphatidyl-



Ser-Ser-Ser-Val-Asp-Gly-Phe-Lys-Ala-Asn-
5 10
Leu-Val-Phe-Lys-Glu-Ile-Glu-Lys-Lys-Leu-
15 20
Glu-Asp-Glu-Gly-Glu-Gln-Phe-Val-Lys-Lys-
25 30
Ile-Gly-Gly-Ile-Phe-Ala-Phe-Lys-Val-Lys-
35 40
Asp-Gly-Pro-Gly-Gly-Lys-Glu-Ala-Thr-Trp-
45 50
Val-Val

Figure 1 - Amino-terminal amino acid sequence and phenylthiohydantoin-amino acid yields of the nonspecific PLEP from bovine liver.

NONSPECIFIC PLEP	D	G	P	G	G	K	E	A	T	W	V	V
PC-SPECIFIC PLEP	D	N	P	G	G	Q	I	P	S	W	V	I

Figure 2 - Alignment of the nonspecific PLEP (residues 41-52) to the phosphatidylcholine-specific PLEP (residues 177-188). Identical residues are enclosed within boxes.

choline-specific PLEP (13) gave a score of 2.25 standard deviations above the scores of randomized sequences.

An inspection of the sequences revealed that the RELATE score is caused by a limited stretch of homology between residues 177-188 in the phosphatidylcholine-specific PLEP and residues 41-52 in the nonspecific PLEP (Fig. 2). It is possible that this may represent a functionally important part of the molecule, which has a similar structure in the two exchange proteins.

ACKNOWLEDGEMENTS

The expert technical assistance of Mr K. Andersson, Mr. T. Fridberger and Ms I. Sjöquist is gratefully acknowledged. This study was supported by a grant from the Swedish Medical Research Council.

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